Preliminary Amendments to a continuation of application PROTEIN STANDARD FOR ESTIMATING SIZE AND MASS

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Art Unit: 1743

**a.) Introductory Comments**

This is a continuation of application (Application Number: 10/068,663) filed on February 6, 2002. The claims 1 to 20 are cancelled. The new claims 21 to 36 are being submitted as part of this CPA.

1. In claim 32 (new), the reference is changed to refer only one claim according to suggestion for claim 11 (cancelled). The detection assay of making the protein standard must be same as the detection assay of using the protein standard in the disclosed application to make the amount estimation accurate.

2. In claim 25 (new), the phrase "where first container means" is changed to "therein at least one container means" according to suggestion for claim 6 (cancelled).

In claim 29 (new), the phrase "combining the polypeptides that each has different" is changed into "combining the polypeptides such that each has different" according suggestion for claim 10 (cancelled).

3. The present invention is not obvious over Novagen catalog (submitted in the Information Disclosure Statement on June 24, 2003) in view of both Hartley (US patent no. 5,834,201, also submitted in the IDS filed June 24, 2003) and New England Biolabs catalog (also submitted in the IDS filed June 24, 2003).

Novagen catalog (page 239) teaches molecular weight standards for protein that are used for estimating size of the sample protein. It also claims that "the known mass of each Perfect Protein™ Markers band also enables estimation of concentration of sample proteins" (page 239 lines 11-14 of column 1). In order to estimate the concentration of sample protein, it provides "50 ug per band except for 100 ug of the 50 kDa band for a 2X reference" (page 239 lines 2-3 and 8-10 of column 2). From these claims, each of 35, 75, 100, and 150 kDa bands of 15-150 kDa Perfect Protein™ Markers should have same

amount of protein. However the Coomassie blue staining intensities of these bands are all different on the bottom-left figure on page 239. If the 50 kDa and 35 kDa bands represent 2X quantity difference, 35 kDa and 75 kDa bands or 35 kDa and 150 kDa bands should represent 3 to 5X quantity differences on same figure on page 239. If one skilled in the art uses the 35 kDa band as reference, 500% less protein may be calculated than using the 150 kDa band as reference. Similar conclusion can be obtained by comparing the band intensities on its figure with their quantities of the 10-225 kDa Perfect Protein™ Markers. In contrast, there are less than 10% variations between the staining intensities and their amounts of different bands in the disclosed application. Therefore the claims on Novagen catalog is just like the names of its Perfect Protein™ Markers. They are nothing more than exaggerated advertisements than serious scientific publications. In addition, Novagen catalog only provided two different amounts of proteins which are not useful in accurate quantity estimation. The description under the title of Perfect Protein™ Markers on Novagen catalog page 239 says "Precisely sized, conveniently spaced for accurate protein size determination". This description truly states that the Perfect Protein™ Markers are for size determination and not for accurate protein quantification. Novagen catalog does not teach the disclosed application.

Hartley (US patent no. 5,834,201) and the New England Biolabs catalog teach DNA ladders that allow one to determine both size and amount of an unknown DNA sample. However DNA and proteins have different chemical compositions, dye-binding properties, and serve different functions in biological systems. Hartley and New England Biolabs do not teach the disclosed application.

Now the question is whether it is obvious to combine the Novagen catalog with both Hartley and New England catalog teachings (or other similar teachings on DNA markers) to make the quantitative protein standard in the disclosed application for one of ordinary skill in the art at the time the invention was made.

The applicant believes the claimed invention is not obvious for one of ordinary skill in the art at the time the invention was made with combination of these prior arts. 1). One of ordinary skill in the art may try to extend the teachings by Hartley and New England Biolabs catalog using the method of making DNA ladders to the protein ladder taught by Novagen catalog. One will find it is impossible to make the desired protein

ladder. First, the molecular weight of DNA is exactly proportional to its length in base pairs regardless of its base composition (DNA has only four base compositions A, T, G, and C. A/ T base pair has exactly same molecular weight of 288 as G/ C base pair excluding riboses). The molecular weight of protein may not be exactly proportional to its length in amino acid since larger amino acid such as Tryptophan has higher molecular weight of 228 and smaller amino acid such as Glycine has lower molecular weight of 90. Second, different compositions of same length of DNA bind same amount of dye such as ethidium bromide while different compositions of same molecular weight proteins have different dye binding properties. When a DNA of 6 kb is digested into 3, 2 and 1 kb fragments, their molecular weight ratio will be 3 to 2 to 1. Their quantity (amount) and dye (ethidium bromide) binding ratio will also be 3 to 2 to1. Therefore the DNA ladders taught by Hartley and New England Biolabs are relative easily made. When a protein of 60 kDa is digested into 30, 20 and 10 kDa fragments, their molecular weight ratio will not be exactly 30 to 20 to 10. Their dye (Coomassie blue) binding ratio is not predictable. When the small fragments have higher affinity to a given dye, all three fragments may give similar staining intensities. In most cases these fragments will give different staining intensities that will not fall into exact integers and will not reflect their molecular weight proportionally. It will be difficult or impossible to co-relate their dye binding intensity to their quantity or amount. Therefore protein ladders made this way will be difficult to use or useless in protein quantification. This might be one reason why quantitative protein marker is not made until the disclosed application.

2). One of ordinary skill in the art may also try to extend the teachings by Hartley and New England Biolabs catalog using the method of making the protein ladder taught by Novagen catalog. One will also find it is impossible to make the desired protein ladder. It is observed that same amount of different proteins give different staining intensities by a given staining assay. The staining intensities can be 3 to 5 times different as evidenced by figures on Novagen catalog. It is also observed different proteins with same staining intensity can contain different amount of protein. The amount of protein can be 5 to 10 times different. Because of these observations, people would not think it is possible to make the invention. For example, when 10, 5, and 3 ug of different proteins are mixed together. The ratio of their staining intensities by a given assay may not be 10:5:3. It may

be 3:5:10 when the third protein stains strongest and the first protein stains weakest. It may also be 20:5:1 when the first protein stains strongest and the third protein stains weakest. It is almost impossible to obtain a ratio of 10:5:3 which reflect the amount of these proteins. Different protein has different amino acid composition. Different amino acid composition gives different staining intensity in a given assay when same amount of protein is used for different proteins. These experimental observations teach away from the disclosed application. So many different staining intensities are produced by same amount of proteins on figures of Novagen catalog page 239, it is clear that more different and unpredictable staining intensities will be produced by different amount of proteins. Same amount of proteins will give different staining intensities (as on Novagen catalog) and different amounts of proteins may give similar staining intensity. It will be extremely difficult to co-relate the staining intensities with the amounts of proteins. The protein ladder made this way will be useless in protein quantification. Many publications showed that people are using laborious ways to determine the protein quantity. The facts that the claimed invention is not a simple combination of previous arts and that those killed in the art use only the more laborious and costly method to determine their protein amount indicate the application is not obvious.

3). Teaching by Hartley ('201) was first filed on October 28, 1993. The earliest Novagen catalog the applicant has is from 1996 which contains the same 15-150 kDa Perfect Protein™ Markers as recent 2005 Novagen catalog. Coomssie Blue was used for protein staining after electrophoresis about 40 years ago (Groth et al, Biochim. Biophys. Acta 71, 377, 1963). Hartley was with Life Technologies Inc. which later became part of Invitrogen. All these companies (Life Technologies, Invitrogen, Novagen and New England Biolabs) have many scientists. They all actively work on protein markers as evidenced by their publications and catalogs. Because of obvious advantages of saving labor and cost with the claimed invention, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for so many years indicates the claimed invention is not obvious.

4. The present invention is not obvious over Hartley (US patent no. 5,449,758, submitted in the Information Disclosure Statement on June 24, 2003) in view of both

Hartley (US patent no. 5,834,201, also submitted in the IDS filed June 24, 2003) and New England Biolabs catalog (also submitted in the IDS filed June 24, 2003).

Hartley ('758) teaches of a protein size marker ladder that comprises at least three polypeptide fragments of different size. Hartley fails to teach that each of the different size polypeptides in the ladder is present at different amount from any of the other polypeptides. Hartley fails to teach that each of the different size polypeptides in the ladder will present different staining intensities that represent different amounts of proteins. Hartley ('758) does not teach the claimed invention.

For teachings of Hartley ('201) and the New England Biolabs catalog, please see previous paragraphs in this Preliminary Amendments. Hartley ('201) and the New England Biolabs catalog do not teach the claimed invention.

Now the question is whether it is obvious to combine Hartley ('758) with both Hartley ('201) and New England catalog teachings to make the quantitative protein standard in the patent application for one of ordinary skill in the art at the time the invention was made.

The applicant believes the claimed invention is not obvious for one of ordinary skill in the art at the time the invention was made with combination of these prior arts. 1). Because of advantages of DNA size and amount ladders taught by Hartley ('201) and New England catalog, one of ordinary skill in the art may try to use the method taught by Hartley ('758) to make a size and quantity protein marker. One will find it is impossible to make the desired protein marker. The protein ladder taught by Hartley ('758) is "a collection of polypeptide fragments obtained by the partial cleavage of a polypeptide--" (lines 2-3 of claim 1 of Hartley '758). Suppose a polypeptide is partially cleaved into three polypeptide fragments, the quantity of each cleaved polypeptide is not fixed. Under one cleavage condition, the largest polypeptide fragment contains most protein. Under another cleavage condition, the medium sized polypeptide fragment contains most protein. Under still another cleavage condition, the smallest polypeptide fragment contains most protein. Each polypeptide produced under each of these conditions will give different staining intensities. In addition, a given amino acid at N-terminus, C-terminus or at the center of a polypeptide may bind dye differently. This will produce added staining intensity variations. Hartley et al (US patent no. 4,403,036) teaches a

DNA ladder produced by partial cleavage of a plasmid DNA. Partial cleavage of DNA cannot make size and quantity DNA standard, Hartley ('201) was awarded for complete digestion of one or more nucleic acids. Similarly partial cleavage of polypeptide cannot make size and quantity protein marker. Protein size and quantity standard is not made until the disclosed invention. The disclosed invention was made without using partial or complete cleavage of polypeptides.

2). Next question is if one of ordinary skilled in the art can make protein size and quantity marker by complete cleavage of polypeptides taught by Hartley ('758). The answer is clearly no. All polypeptides taught by Hartley ('758) are comprised of "multiple polypeptide repeats of identical sequence" (lines 3-4 of claim 1 of Hartley '758). They will be cleaved into one size when the cleavage is complete. Complete cleavage of a polypeptide into polypeptide fragments with different amino acid compositions was discussed in previous paragraphs. These prior arts or any combination of them do not teach the disclosed invention.

3). Teaching by Hartley ('201) was first filed on October 28, 1993. Teaching by Hartley ('758) was filed on December 2, 1993. It has been about fifteen years since these teachings are filed. Because of obvious advantages of saving labor and cost with the claimed invention, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for so many years indicates the claimed invention is not obvious over these teachings or any combination of them.

5. Chatterjee et al (US patent no. 6,703,484) teaches polypeptides of different molecular weights, wherein one polypeptide is stained with a first dye and all remaining polypeptide are stained with a second dye wherein the first and the second dye are not the same. In other words, Chatterjee et al teaches polypeptides stained with different dyes (colors). They can be used as protein markers pre-stained with different colors. Chatterjee et al does not teach the claimed invention. The applicant appreciated the prior art provided by the examiner and made note of it.

6. The applicant devised new principles of operation to make the invention possible: (1) the absolute amount of each protein is not relevant in the quantitative protein standard. (2) The relative staining intensity of each protein will represent a quantity of a standard protein such as bovine serum albumin (BSA). In other words, the relative amount of

protein obtained from the relative staining intensity does not reflect the amount of each protein in the quantitative protein standard but represent the relative amount of protein of the standard protein such as BSA. (3) The staining assay used to prepare the quantitative protein standard should be same as the staining assay of using the protein standard to make the quantification reliable and consistent. This means that if the quantitative protein standard is made by Coomassie Blue staining, the standard should only be used by Coomassie Blue staining assay. Using it in silver staining assay will not be accurate in estimating the protein amount. Similarly if UV spectrometer is used to quantify the proteins in the quantitative protein standard, it cannot be used in either Coomssie Blue staining or silver staining to accurately estimate the amount of a sample protein. (4) Same experimental procedures should be used for estimating the amount of proteins in making the quantitative protein standard and using the standard. The amount of the proteins in the quantitative standard is estimated by a polyacrylamide gel electrophoresis followed by a detection assay such as Coomassie Blue staining since the standard is to be used in the same procedures (claims 26, 29 and 37). Electrophoresis on a polyacrylamide gel before a detection assay to estimate the amount of protein in the protein standard is a critical step. Substances such as detergents, buffers, salts and reducing agents that may affect detection are separated from polypeptides, run off the gel during electrophoresis and washed out during staining and de-staining procedures. The quantitative protein standard can be made accurate only with these procedures. Estimating protein amount of quantitative protein standard without electrophoresis step will not be accurate even same assay is used in using the standard. Each protein contains different detergents, buffers, salts and reducing reagents. These substances may affect quantity estimation with a given detection assay.

The quantitative protein standard was made possible only after all of these new principles of operation were used. It is not possible to practice the disclosed invention without any of these new principles of operation. These new principles of operation are not taught by prior arts or combination of them. Involvement of new principles of operation indicates the disclosed invention is not obvious.

7. The invention addresses a long-felt, long-existing, but unsolved need. The applicant has been working on protein sizing and quantification for many years. It was

always painful to use the laborious method taught by Fishbein (Anal. Biochem., 46,388-401, submitted in the IDS filed June 24, 2003). This laborious and costly method is still used today in many academic and industrial labs as revealed by Houghton et al (US patent no. 6,168,946, cited in the Notice of Reference Cited mailed on March 28, 2003). Therefore solution for the long-felt, long existing, but unsolved need further indicates the application is not obvious.

In conclusion, the disclosed invention involves new principles of operation and it is not obvious over any previous arts or combination of them. Therefore Claims 21, 26 and 29 are patentable. Claims 22-25, 27-28 and 30-38 are dependent on Claims 21, 26 and 29. They are provided for fully disclosure of the invention, therefore they are also patentable. If the examiner agrees but does not feel that the present claims are technically adequate, applicant respectfully requests that the examiner write acceptable claims pursuant to MPEP 707.07(j).